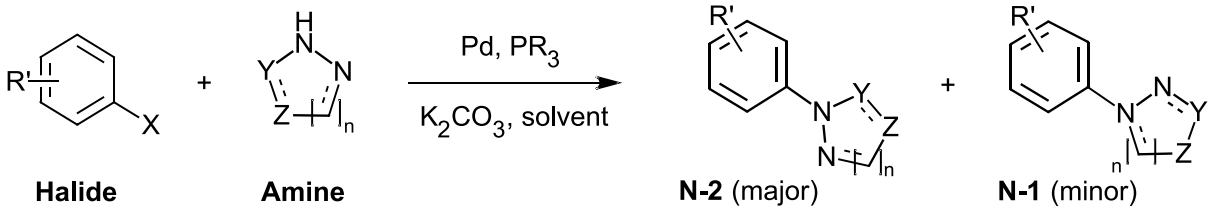
CatSci
BEYOND PROCESS CHEMISTRY

Process Development Case Study
WORK-UP
Design of a Washing Regime to Purge a Regioisomer

INTRODUCTION
An optimised palladium-catalysed **Buchwald-Hartwig amination** reaction was found to **reliably produce** the hetero-biaryl product with a regioisomeric ratio in solution of **92:8** in favour of **the desired N-2 isomer** at end of reaction (EOR).



Halide **Amine** **N-2** (major) **N-1** (minor)

Crystallisation using the prevailing conditions resulted in an **unacceptable level** of 1.5% of N-1 isomer still remaining in the isolated drug substance. Since the drug substance specification required <0.3% of N-1 isomer the challenge was to **improve the purity** further on work-up by **removing the undesired isomer** with **minimum loss** of the requisite N-2 **product**.

KEY OBSERVATION
The aqueous wash after the cross-coupling reaction removed excess base and inorganic salts. Analysis showed that there had also been a **preferential partitioning of the unwanted N-1** isomer into the **aqueous wash**. A significant improvement in the isomer ratio to 95:5 for the organic phase was observed, with a concomitant ratio of 67:33 in the aqueous phase; **loss of product** to the aqueous phase was **7%** (**Table 1**).

	At EOR	After wash #1		After wash #2		After wash #3	
Temp. (°C)	**Ratio***	**Ratio***	**% loss (total)†**	**Ratio***	**% loss (total)†**	**Ratio***	**% loss (total)†**
25	92 : 8	95 : 5	18	97 : 3	59	99 : 1	61
80	92 : 8	95 : 5	7	97 : 3	12	99 : 1	18

***** ratio is of N-2 : N-1 isomers in organic phase; **†** % loss is cumulative from all sources

Table 1 – cumulative effects of aqueous washes at low and high temperatures

When **multiple water washes** were carried out, the ratio of N-2 to N-1 improved further to **99:1**. However, this also resulted in partial precipitation of the N-2 product at the interface, leading to **significant loss of product** from the organic phase and thus from the process. This was much **less pronounced at higher temperatures** due to increased solubility (18% loss over three washes at 80 °C versus 60% loss over three washes at 20 °C).

This **observation** showed that the **first wash was more selective** in extracting the undesired N-1 isomer and did not cause the precipitation of the N-2 product at the interface that was observed in following washes. These results were attributed to either the **difference in the ionic strength** and/or **the pH of the initial aqueous wash** that arose following the removal of the dissolved inorganic salts and excess base from the crude reaction mixture, respectively. To test this hypothesis further, the subsequent **washes** were **designed** to imitate the **ionic and basic properties of the first wash**.

DISCUSSION
When **brine washes** were employed as substitutes for simple water washes, the loss of the **desired N-2 isomer** to the aqueous phase was **significantly reduced** over the course of three washes (11% compared to 18%) whilst the preference for extraction of the N-1 isomer over the N-2 isomer was retained (**Table 2**). In addition, **the partial precipitation of product** at the interface observed in the second and third water washes was **no longer observed** when using brine solutions. The individual washes (each 10 relative volumes) were designed with ionic strengths varying from 1-3% w/v sodium chloride to ensure maximum retention of the undesired N-1 isomer.

	At EOR	After wash #1		After wash #2		After wash #3	
Brine		**1% w/v**		**3% w/v**		**3% w/v**	
Phase	**Ratio***	**Ratio***	**% loss (total)†**	**Ratio***	**% loss (total)†**	**Ratio***	**% loss (total)†**
Organic	92 : 8	96 : 4	n/a	97 : 3	n/a	>98 : 2	n/a
Brine	n/a	55 : 45	neg.	65 : 35	3.1	90 : 10	11

* ratio is of N-2 : N-1 isomers in either phase; † % loss is cumulative from all sources
n/a = not applicable; neg. = negligible

Table 2 – cumulative effects of brine washes at 80 °C

To further evolve this concept, **alkaline brine solutions** were also investigated. A basic pH for the second and third washes was achieved by the dissolution of 1 equivalent of K_2CO_3. This was expected to reduce the loss of the desired N-2 isomer in the aqueous layer still further, and indeed this was found to be the case. Accordingly, **loss of the desired N-2 isomer** was limited to a total of only **5%** using such alkaline brine washes, whilst the selectivity for the N-1 isomer remained unaltered at >98:2 (**Table 3**).

	At EOR	After wash #1		After wash #2		After wash #3	
Alkaline brine		**1% w/v**		**3% w/v plus K_2CO_3**		**3% w/v plus K_2CO_3**	
Phase	**Ratio***	**Ratio***	**% loss (total)†**	**Ratio***	**% loss (total)†**	**Ratio***	**% loss (total)†**
Organic	92 : 8	95 : 5	n/a	97 : 3	n/a	>98 : 2	n/a
Brine	n/a	55 : 45	neg.	60 : 40	neg.	70 : 30	5

* ratio is of N-2 : N-1 isomers in either phase; † % loss is cumulative from all sources
n/a = not applicable; neg. = negligible

Table 3 – cumulative effects of alkaline brine washes at 80 °C

EXPERIMENTAL PROCEDURE

All experiments were carried out in a nitrogen filled glove-box using nitrogen degassed solvents. The reactions were carried out on a scale of at least 2 g of starting Halide. The active catalyst was prepared out by heating the Pd pre-catalyst/ligand solution at 60 °C for 30 mins. This was transferred to the other reagents and the reactions were then stirred at 110 °C for 5 hours. The temperature of the reaction mixture (15 volumes) was reduced to 80 °C after completion of the reaction and the aqueous phase (10 volumes, water/brine/alkaline brine) was heated to 80 °C prior to addition. The resulting mixture was stirred for 20 minutes at 80 °C, then allowed to stand for 20 minutes to ensure full phase separation. Brine washes: wash 1 (1% w/v NaCl), washes 2 and 3 (3% w/v NaCl). Alkaline brine washes: wash 1 (1% w/v NaCl), washes 2 and 3 (3% w/v NaCl with 1 eq. K_2CO_3).

SUMMARY

Optimisation of the work-up successfully **improved the regioisomeric ratio** from 92:8 at EOR to >98:2 in solution with minimal loss of yield (~5%). The final crystallisation of the drug substance then resulted in <0.2% of the unwanted N-1 isomer, **within specification**. This was achieved through i) initial **experimental observation**; ii) **correct deduction** of its significance; iii) **quantitative analysis** of the organic and aqueous phases. Thorough understanding of the work-up process enabled successive improvements to be harvested. The **beneficial work-up** was fully **demonstrated** at CatSci (0.5 kg batch, 20 L scale) and subsequently at the customer's **manufacturing facility**.

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